RNA Assay User Guide

For LabChip GX Touch/GXII Touch

CE

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PN CLS140164, Rev. C

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Specifications

Assay Specifications

Table 1. Assay Specifications

Linear Range	25 - 250 ng/µL (Std Sens) 5 - 50 ng/µL (High Sens)
Quantitation Reproducibility	20% CV
Size Range	100 - 6000 nucleotides (suitable for total RNA)
RNA Sample Volume	2 µL of user sample (Std Sens) 6 µL of user sample (High Sens)
Run Time	80 seconds per sample (about 2.5 hours for 96 samples)
Setup Time	Approximately 30 minutes to prepare chip and samples
Number of Samples per Chip Prep	200 samples
Samples per Reagent Kit	5 chip preps

Sample Conditions

Table 2. Sample Conditions

Additives	PerkinElmer recommends that BSA and detergents exceeding 0.05 mg/mL and 0.01% (v/v) respectively in concentration not be used. Higher concentrations can result in chip failure. In addition, non-aqueous solvents are not compatible with LabChip protocols.
Particulates	All sample plates should be spun down prior to analysis. All buffers should be filtered with a 0.22 µm cellulose acetate filter.
Salt Concentration	Total salt concentration must not exceed 10 mM Tris. Higher salt concentrations and different ions may alter performance and reduce assay sensitivity.

Kit Contents

Storage: When not in use, store chips and reagents refrigerated at 4°C. Do not leave chips and reagents unrefrigerated overnight. Store the RNA Ladder at -70°C.

Table 3. RNA Reagent Kit Contents, PN CLS960010

Reagent	Vial	Quantity
RNA Dye Concentrate	Blue	1 vial, 0.5 mL
Chip Storage Buffer	White	5 vials, 1.8 mL each
RNA Gel Matrix	Red	5 vials, 0.510 mL each
RNA Pico Ladder (packaged separately, P/N 760634)[a]	Yellow	2 vial, 0.03 mL each
RNA Marker	Green	1 vial, 0.8 mL
10X RNA Sample Buffer Concentrate	Purple	6 vials, 2.0 mL each

a. Additional RNA Ladder can be ordered separately using Part Number 760634.

Table 4. Consumable Items

Item	Supplier and Catalog Number	Quantity
Spin Filters	Costar, Cat. # 8160	10
Ladder Tubes	Genemate, Cat. # C-3258-1	10, 0.2 mL
Detection Window Cleaning Cloth	VWR, Cat. # 21912-046	1
Swab	ITW Texwipe®, Cat. # TX758B	3
Buffer Tubes	E&K Scientific, Cat. # 697075-NC	10, 0.75 mL

Table 5. RNA LabChips

Item	Catalog Number
DNA 5K/RNA/CZE Chip for use with GX Touch/GXII Touch HT	Cat. # 760435
DNA 5K/RNA/CZE Chip for use with GX Touch/GXII Touch 24	Cat. # CLS138949

Safety Warnings and Precautions

WARNING!

For Research Use Only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

CAUTION

We recommend that this product and components be handled only by those who have been trained in laboratory techniques and that products are used in accordance with the principles of good laboratory practice. As all chemicals should be considered potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

WARNING!

Dye Concentrate contains DMSO. S24/25: Avoid contact with skin and eyes.

Preparation Procedures

Additional Items Required

- 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent).
- 70% isopropanol solution in DI water.
- DEPC treated water (nuclease-free)
- PCR cap strips
- Bio-Rad Hard-Shell® 384-well Skirted PCR Plates, Cat # HSP-38XX (recommended).
- PerkinElmer Hard-Shell thin-wall 96-well skirted PCR plate (blue), Cat # 6008870 (recommended).

Note: *Allow the chip and reagents to equilibrate to room temperature for 20-30 minutes before use.*

Preparing the Gel-Dye Solution

Notes: *The Dye Solution contains DMSO and* ***must be thawed completely*** *before use.*

The dye is light sensitive. ***Do not expose the Dye solution or Gel-Dye to light for any length of time.*** *Keep the prepared Gel-Dye solution in the dark.*

1 Vortex the thawed RNA Dye Concentrate (blue cap 🔵) for 10 - 15 seconds before use.

2 Transfer 90 µL of RNA Dye Concentrate (blue cap 🔵) to one vial of RNA Gel Matrix (red cap 🔴). Vortex until the solution is well mixed and transfer to a spin filter.

3 Centrifuge at 9300 rcf for 10 minutes at room temperature.

4 Discard filters, label and date the tubes.

5 Store in the dark at 4°C. Use within 3 weeks.

Note: *The volume of Gel-Dye solution prepared is the required amount for one HT (High-throughput) or two LT (Low-throughput) chip preps.*

Preparing the RNA Samples and RNA Ladder

1. Prepare sample buffer by adding 620 µL RNA Sample Buffer Concentrate (purple cap ●) to 5580 µL DEPC treated water.

   Note: *This volume is enough for one full 96-well plate run (includes the samples, ladder and buffer tube.You may adjust accordingly for partial plates. Sample Buffer is stable after dilution, but to avoid RNase contamination, sample buffer should be prepared fresh.)*

2. Allow the RNA Ladder (yellow cap ●) to thaw on ice. (It is recommended that you aliquot the RNA Ladder to 4-µL aliquots for individual use.)

3. Pipette 4 µL of RNA Ladder into the provided 0.2 mL Ladder Tube and cover.

4. Pipette 2 µL of sample into individual wells of a microtiter plate or into RNase-free microcentrifuge tubes. (If running the High Sens assay, pipette 6 µL of each sample.) Samples in microtiter plates should be covered with PCR cap strips. Foil is not recommended as the adhesive may contaminate the samples. If diluting the samples, use nuclease-free water.

5. Heat samples and ladder for 2 minutes at 70°C.

6. Snap cool the ladder and samples by immediately placing the tube/plate on ice for 5 minutes.

7. Add 46 µL of prepared sample buffer (prepared in Step 1) to each sample. (If running the High Sens assay, add 19 µL of sample buffer to each sample). Mix by pipetting up and down a few times. Avoid creating air bubbles. Cover with PCR cap strips and spin down the plate at 1250 rcf for 1 minute at room temperature to remove air bubbles.

8. Add 96 µL of prepared sample buffer to the RNA Ladder. Ensure there are no air bubbles in the Ladder Tube.

9. Insert the Ladder Tube into the ladder slot on the LabChip GX Touch/GXII Touch instrument (see Figure 1 on page 8).

Preparing the Buffer Tube

1. Add 750 µL of RNA Sample Buffer solution to the 0.75 mL Buffer Tube provided with the reagent kit. Ensure there are no air bubbles in the Buffer Tube.
2. Insert the Buffer Tube into the buffer slot on the LabChip GX Touch/GXII Touch instrument (see Figure 1).

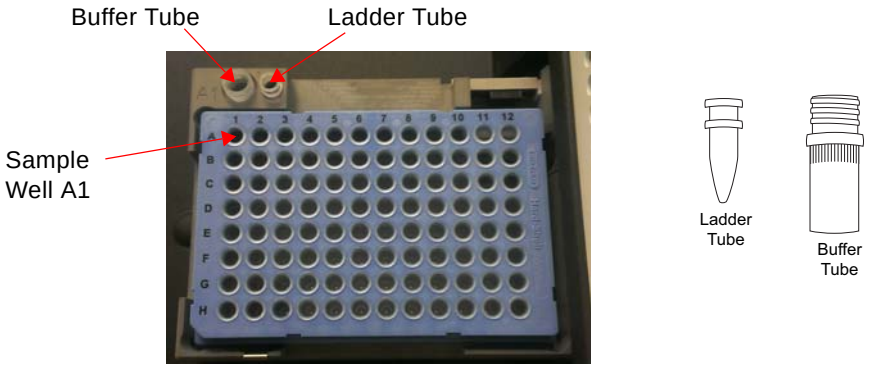

Figure 1. Locations of the Buffer Tube and Ladder Tube in the GX Touch/GXII Touch instrument.

Preparing the Chip

1. Allow the chip to come to room temperature and remove the foil cover from chip wells.
2. Use a pipette tip attached to a vacuum line to thoroughly aspirate all fluid from the chip wells (see Figure 2). For more details on how to set up a vacuum line see page 32.

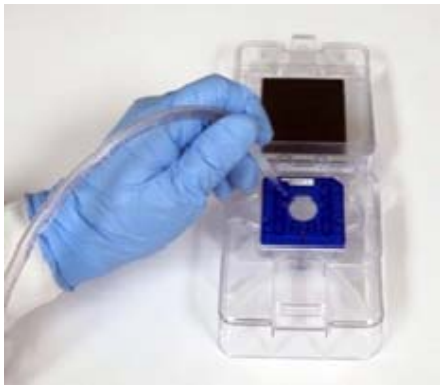

Figure 2. Using a vacuum to aspirate the chip wells is more effective than using a pipette.

3 Rinse and completely aspirate each active chip well (1, 3, 4, 7, 8 and 10) twice with nuclease-free water. Do not allow active wells to remain dry.

4 If any water spills onto the top and bottom chip surfaces during rinsing, aspirate using the vacuum line. DO NOT run the tip over the central region of the detection window. Use the provided Detection Window Cleaning Cloth dampened in water (Milli-Q® or equivalent) or alcohol to clean the chip detection window as needed.

5 Using a reverse pipetting technique, add Gel-Dye solution to chip wells 3, 7, 8 and 10 as shown in Figure 3 (Low-throughput) or Figure 4 (High-throughput).

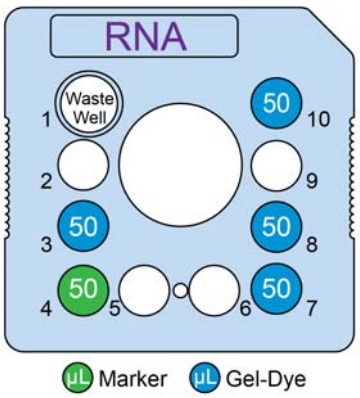

Figure 3. Low-throughput chip preparation.

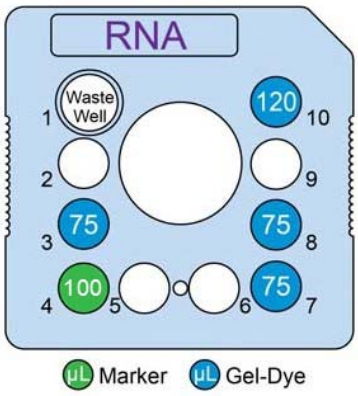

Figure 4. High-throughput chip preparation.

6 Add 50 µL (Low-throughput) or 100 µL (High-throughput) of RNA Marker (green cap ●) to chip well 4 as shown in Figure 3 or Figure 4.

Note: *The marker well may need to be replenished if the chip is in idle mode on the instrument for an extended period of time.*

7 Make sure the rims of the chip wells are clean and dry.

8 **IMPORTANT:** Ensure chip well 1 (waste well) is empty before placing the chip into the instrument.

Inserting a Chip into the LabChip GX Touch/GXII Touch Instrument

1. Check that the sample plate, Buffer Tube, and Ladder Tube are placed on the instrument properly.
2. Remove the chip from the chip storage container and inspect the chip window. Clean BOTH sides of the chip window with the PerkinElmer-supplied clean-room cloth dampened with a 70% isopropanol solution in DI water.
3. Touch the *Unload Chip* button on the *Home* screen.

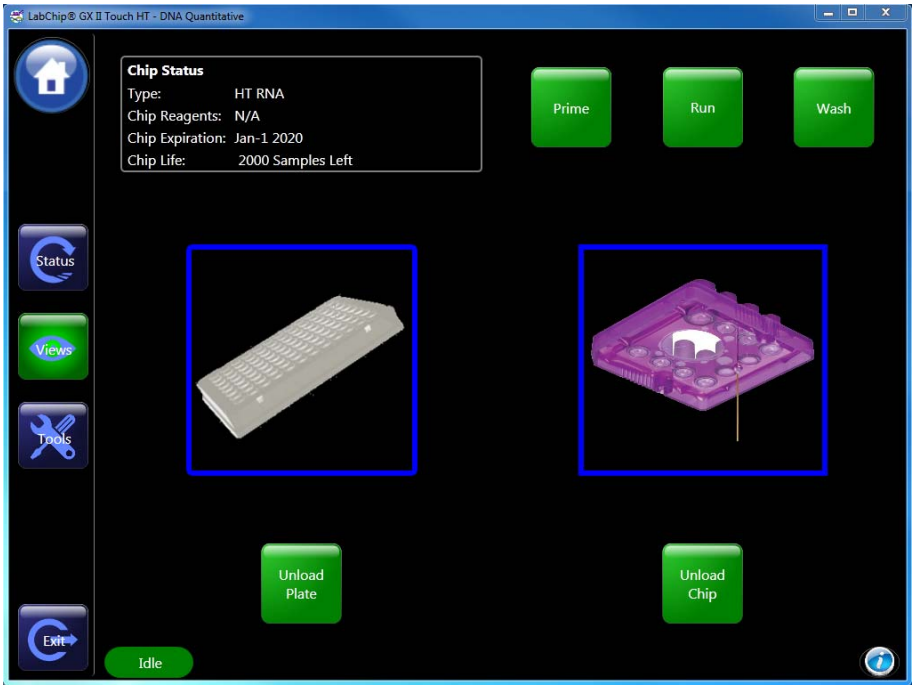

Figure 5. *Home* screen.

4. Insert the chip into the LabChip GX Touch/GXII Touch instrument (Figure 6) and close the chip door securely.

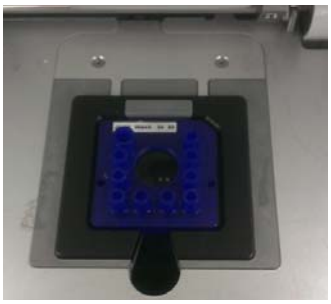

Figure 6. Chip in the LabChip GX Touch/GXII Touch instrument.

5 Touch the *Load Plate* button on the *Home* screen (Figure 5) to retract the sample plate and send the sipper to the Buffer Tube.

Note: *Do not keep the chip door open for any length of time. Dye is sensitive to light and can be photobleached.*

6 The *Assay Choice* window will appear (Figure 7). Touch the desired assay and then touch *OK*.

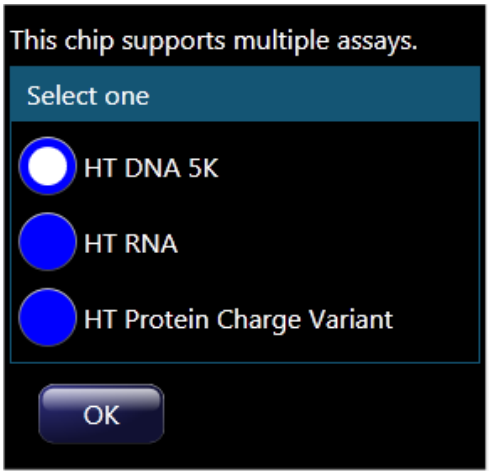

Figure 7. *Assay Choice* window.

Notes: *If performing multiple runs in a day, in between chip preparations the chip should be washed using the instrument and Chip Storage buffer as described in* *"Refreshing the Gel-Dye Solution" on page 15**.*

Be sure to periodically clean the O-rings on the top plate of the chip interface on the LabChip GX Touch/GXII Touch. Use the provided lint-free swab dampened with water to clean the O-rings using a circular motion. Allow the O-rings to dry before inserting a chip.

Running the Assay

Note: *Chips can be primed independently from running assays. Touch the Prime button on the Home screen.* ***Make sure the Buffer Tube is placed on the instrument.***

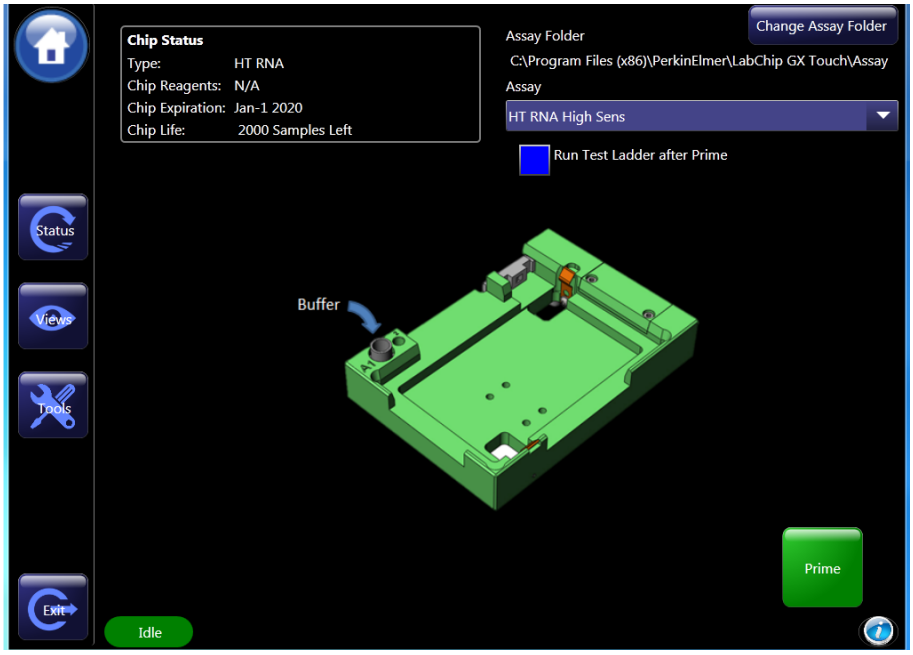

Figure 8. Chip priming screen.

1 Touch the *Run* button (see Figure 8).

2 Select the appropriate assay type (see Figure 9). For RNA assays the appropriate assay types are:

- **RNA Std Sens:** For detection of RNA in the 25 - 250 ng/µL range.
- **RNA High Sens:** For detection of RNA in the 5 - 50 ng/µL range (requires a higher sample volume).

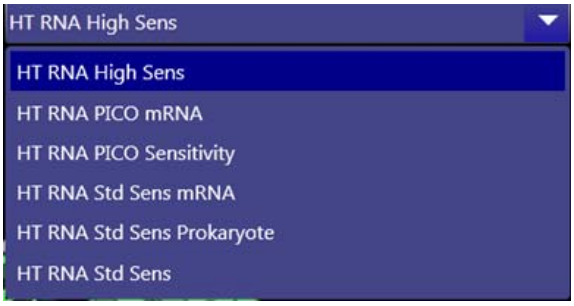
HT RNA High Sens
HT RNA High Sens
HT RNA PICO mRNA
HT RNA PICO Sensitivity
HT RNA Std Sens mRNA
HT RNA Std Sens Prokaryote
HT RNA Std Sens

Figure 9. *Assay Choice* window.

3 Select the plate name, well pattern, and whether to read wells in columns or rows. Select number of times each well is sampled under *Adv. Settings* (Figure 10). Touch the *green arrow* button.

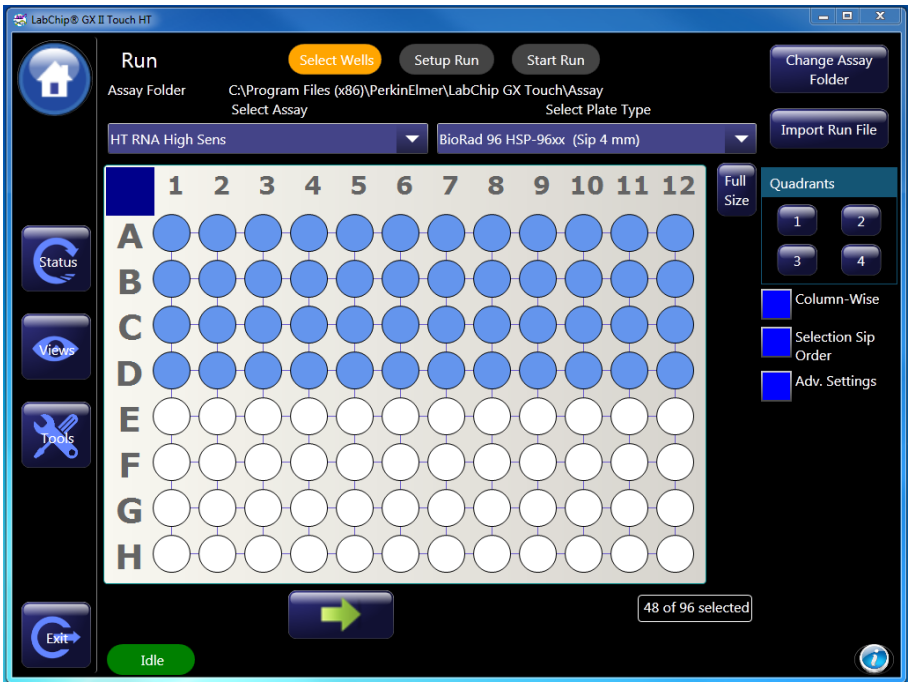

Figure 10. Selecting wells.

4 In the *Setup Run* tab, select the operator name, the option to read barcode, the destination of the file, the inclusion of sample names, expected peaks, and excluded peaks and the filename convention. Select *Auto Export* to export results tables automatically (Figure 11). Touch the *green arrow* button.

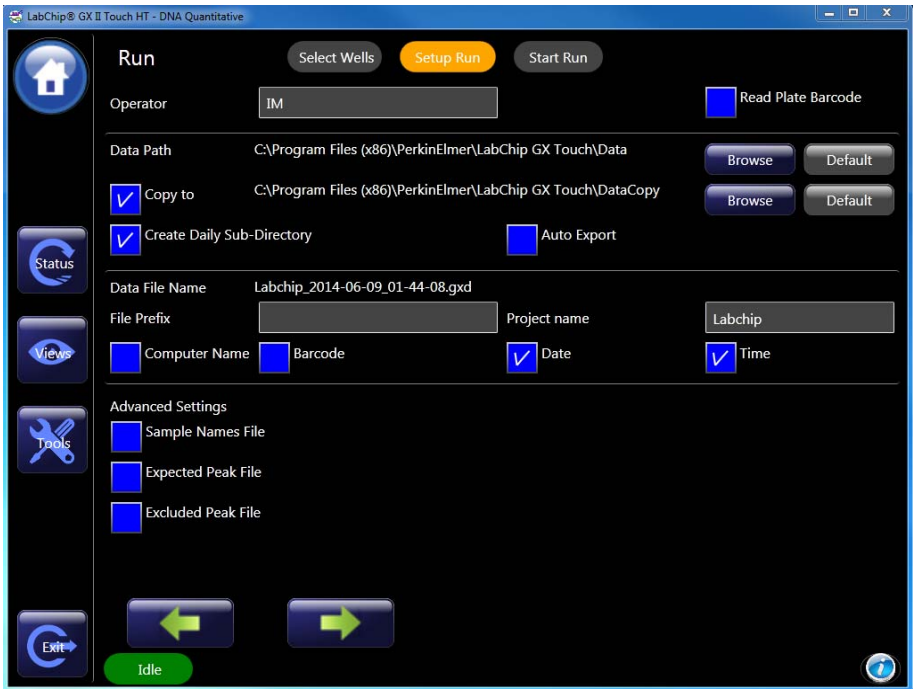

Figure 11. Run setup screen.

5 Touch *Start* to begin the run (Figure 12).

Figure 12. Starting a run.

Refreshing the Gel-Dye Solution

After one full 96-well plate has been run (HT chip prep) or if the Gel-Dye solution was placed in the chip more than 3 hours ago (LT or HT chip prep), we recommend the user refresh the Gel-Dye solution in well 10. To do this, touch the *Unload Chip* button on the *Home* screen to open the instrument door. Remove the residual Gel-Dye solution in well 10, either by pipette or by vacuum aspiration.

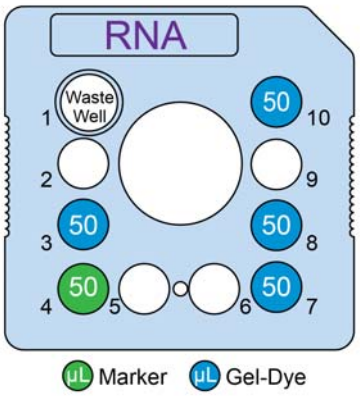

Figure 13. Low-throughput chip preparation.

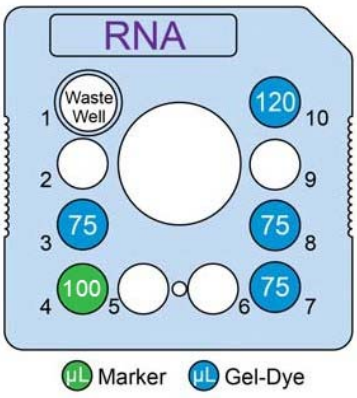

Figure 14. High-throughput chip preparation.

The well does not need to be washed or rinsed. After the previous Gel-Dye solution has been removed, add 50 µL (LT) or 120 µL (HT) of fresh, unused Gel-Dye into well 10. (This could be the leftover Gel-Dye mixture from an earlier filtered Gel-Dye prep). Only well 10 needs to be refreshed, you may leave the remaining other reagents in the chip wells. Once well 10 has been refreshed, close the instrument door securely, then start the next run.

Storing the Chip

After use, the chip must be cleaned and stored in the chip container. The procedure below can be conducted the following day when running overnight.

1 Place the chip into the plastic storage container. The sipper should be submerged in the fluid reservoir.

2 Remove the reagents from each well of the chip using vacuum.

3 Each active well (1, 3, 4, 7, 8 and 10) should be rinsed and aspirated twice with water (Milli-Q® or equivalent).

4 Add 120 µL of Storage Buffer (white cap ◯) to the active wells.

5 Place the chip in the LabChip GX Touch/GXII Touch instrument and touch the *Wash* button in the upper right corner in the *Home* Screen.

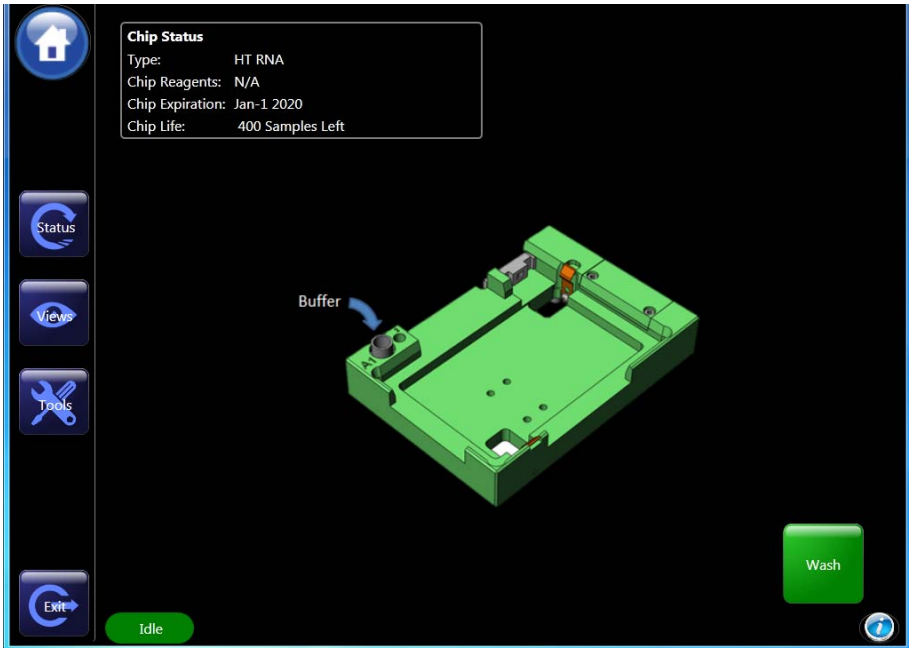

Figure 15. *Wash* screen.

6 Remove the chip from the instrument and place it in the plastic storage container. Add an additional amount of Storage Buffer to well 1.

7 Cover the wells with Parafilm® to prevent evaporation and store at 4°C. Storage of a chip with dry wells may cause it to become clogged.

Chip Cartridge Cleaning

1 **Daily**

a Inspect the inside of the chip cartridge and O-rings for debris.

b Use the provided lint-free swab dampened with water (Milli-Q® or equivalent) to clean the O-rings using a circular motion. If the O-rings stick to the chip or a pressure leak is detected, perform the more extensive monthly cleaning procedure.

2 Monthly

a To reduce pressure leaks at the chip interface, clean the O-rings frequently. Remove the O-rings from the top plate of the chip interface on the LabChip GX Touch/GXII Touch instrument. Soak O-rings in water (Milli-Q® or equivalent) for a few minutes. Clean the O-ring faces by rubbing between two fingers. Wear gloves.

b To reduce the occurrence of current leaks, clean the chip interface frequently. Clean the top plate of the chip interface using the provided lint free swab dampened with water (Milli-Q® or equivalent).

c Allow the O-rings and chip interface to air dry. Reinsert the O-rings into the chip cartridge.

Results

RNA Ladder Result

The electropherogram of a typical RNA ladder is shown in Figure 16.

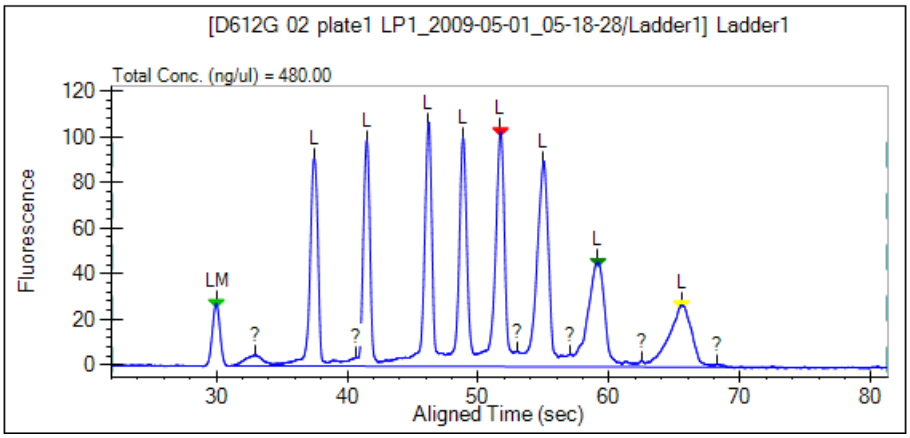

Figure 16. Typical RNA ladder.

RNA Eukaryote Sample Result

The electropherogram for typical total RNA samples is shown in Figure 17. Your results may vary depending on the type of total RNA.

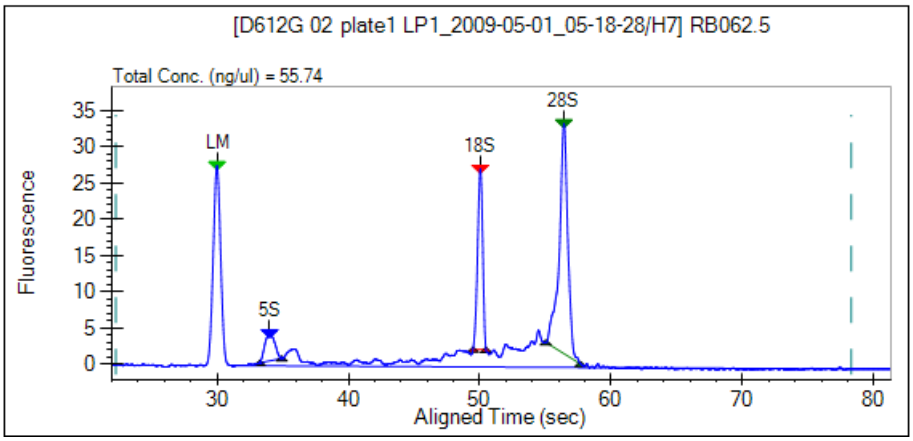

Figure 17. Electropherogram for a typical total RNA sample.

RNA Prokaryote Sample Result

The electropherogram for typical Prokaryote Total RNA samples is shown in Figure 19. Your results may vary depending on the type of total RNA.

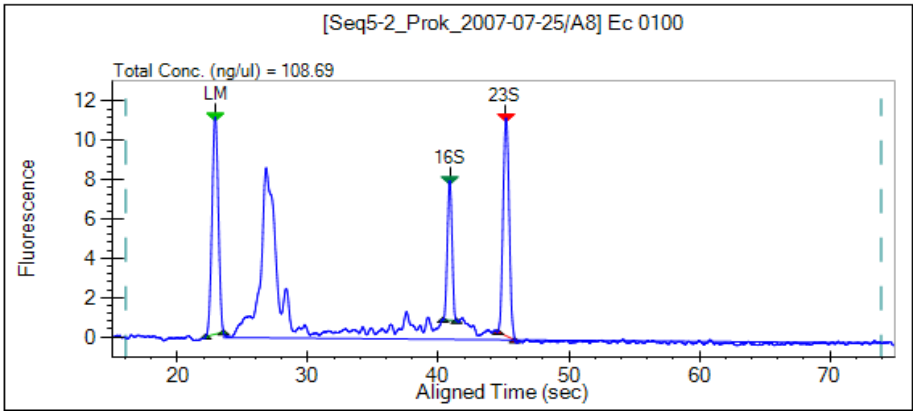

Figure 18. Electropherogram for a typical prokaryote RNA sample.

RNA mRNA Sample Result

The electropherogram for a typical mRNA sample is shown in Figure 19. Your results may vary depending on the type and concentration of mRNA.

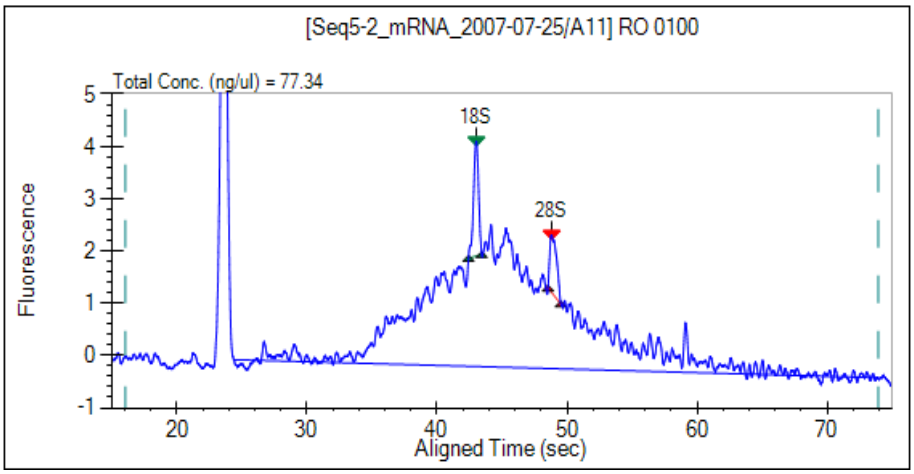

Figure 19. Electropherogram for a typical prokaryote RNA sample.

Troubleshooting

Note: *Some of the data examples shown in this section were generated with assays other than the assay described in this user guide.*

Symptom: No ladder or sample peaks but marker peaks detected.

Note: *The lower marker peak height will most likely be greater than normal height.*

Possible causes:

1 Air bubble in sipper introduced during chip priming.

What to do:

1 Reprime the chip. See “LabChip Kit Essential Practices” on page 26 for instructions on how to reprime the chip.

Symptom: Missing sample, ladder *and* marker peaks.

Possible causes:

1 Clog in sipper or marker channel of chip.

What to do:

1 Reprime the chip. See “LabChip Kit Essential Practices” on page 26 for instructions on how to reprime the chip.

Symptom: Ladder detected but no sample peaks.

Possible causes:

1 The sipper is not reaching the sample due to low sample volume in the well of the plate.

2 If the missing sample peaks occurred only in a few wells of the plate, check those wells for air bubbles.

3 The sipper is not reaching the sample due to an incorrect capillary height setting or incorrect plate definition.

4 If the plate has been uncovered for some time, sample evaporation might have occurred.

5 Debris from the sample or sample prep is clogging the sipper.

What to do:

1 Add more sample to the well.

2 Manually insert a larger volume pipette tip (~100 µL) into the sample well and dislodge the bubble. Rerun these sample wells.

3 Check the plate definitions.

4 Check the sample wells, especially around the edge of the plate where evaporation is fastest, and make a fresh plate if volumes are low.

5 If you suspect there may be debris in your samples, spin the sample plate down in a centrifuge (e.g. 3000 rcf for 5 minutes). Unclog the sipper by repriming the chip. See “LabChip Kit Essential Practices” on page 26 for instructions on how to reprime the chip.

Symptom: No ladder peaks but sample peaks and marker peaks are present.

Possible causes:

1 Low or no ladder volume in the Ladder Tube.

What to do:

1 Add more ladder to the Ladder Tube and restart the run. Recommended standard ladder volume is 120 µL (minimum volume is 100 µL).

Symptom: No marker peaks but sample peaks are present.

Possible causes:

1 No marker added to chip well 4.

2 If there is marker solution in chip well 4, the problem may be due to a marker channel clog.

What to do:

1 This may be due to not filling marker well or chip remaining idle on instrument for extended period of time. Add or replenish the marker solution in the chip using the following procedure:

- Touch the *Unload Chip* button on the Home screen to open the chip door.
- Return the chip to the chip container ensuring the sipper is immersed in fluid.
- Thoroughly aspirate all fluid from chip well 4 using a vacuum line.
- Ensure that chip well 4 is rinsed and completely aspirated twice with water (Milli-Q® or equivalent).
- Add Marker Solution (green cap ●) to chip well 4.

- Reinsert the chip back into the instrument.
- Restart the run.

2 Perform a marker channel unclogging procedure by repriming the chip. See “LabChip Kit Essential Practices” on page 26 for instructions on how to reprime the chip.

Symptom: Ladder traces show up in the lanes following the ladders (delayed sip).

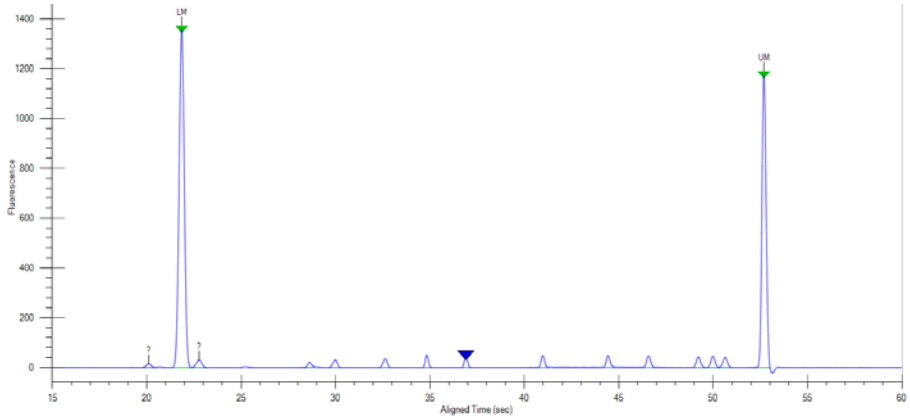

Figure 20. Small ladder peaks in sample well caused by delayed sip.

Possible causes:

1 Separation channel overloaded with sample.

2 Partial clog in the separation channel.

What to do:

1 Lower the starting sample concentration.

2 Reprime the chip. See “LabChip Kit Essential Practices” on page 26 for instructions on how to reprime the chip.

Symptom: Unexpected sharp peaks.

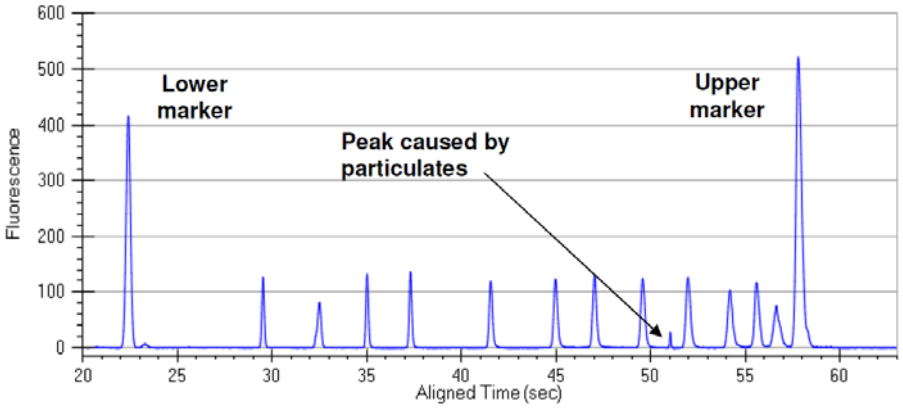

Figure 21. Unexpected sharp peak.

Possible causes:

- Dust or other particulates introduced through sample or reagents.

What to do:

1 Do one or all of the following:

- Replace the 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent) water used for chip preparation.
- Replace the buffer used for sample and reagent preparation.
- Use a 0.22-micron filter for all water and buffers used for chip, sample, and reagent preparation.
- Spin down sample plate to pellet any particulates.

Symptom: Humps in several electropherograms which do not correspond to sample data.

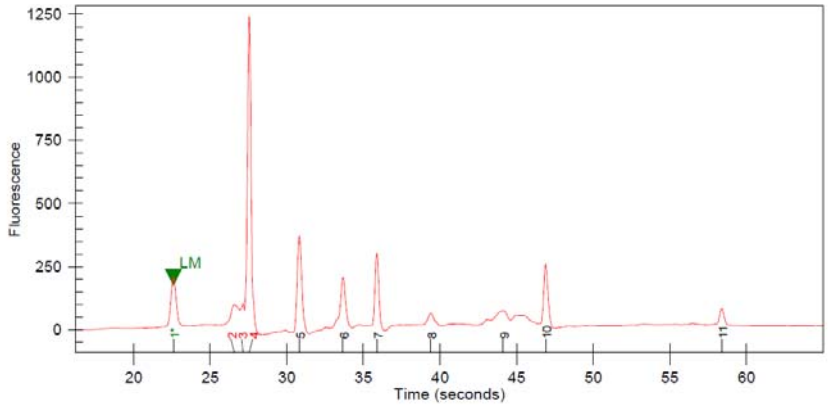

Figure 22. Humps in several electropherograms.

Possible causes:

1 Electrode 7 is dirty and has contaminated the Gel-Dye mixture in well 7.

What to do:

1 Before restarting the run, clean electrode 7. Remove the chip and follow the electrode cleaning procedure. We recommend using the provided swab and isopropanol to manually clean electrode 7.

Symptom: Peaks migrating much faster or slower than expected.

Note: *Some migration time variance between chips or within a plate is considered normal chip performance. All chips are QC tested at PerkinElmer prior to shipment.*

Normal migration time windows for the markers are:

- *RNA Pico Sensitivity Assay Lower Marker: 28 - 33 seconds*

Possible causes:

1 Incorrect Gel-Dye ratio. Migration time is sensitive to dye concentration and peaks will migrate too fast or too slow if the dye concentration in the gel is too low or too high, respectively.

Note: *Excess dye within the separation channel will slow down migration, and less dye in the separation channel will make peaks migrate faster.*

2 Particulates from the samples may be clogging the separation channel (this will slow down migration).

3 Gel-Dye was not primed properly into the chip.

What to do:

1 Prepare a fresh Gel-Dye solution. Wash and reprime the chip with the new Gel-Dye mixture. See “LabChip Kit Essential Practices” on page 26 for instructions on how to wash and reprime the chip.

2 If fast or slow migration is observed repeatedly on a new chip, contact technical support to arrange return of the chip to PerkinElmer. Please send a data file showing the failure along with the return request.

3 Minimize the loading of particulates in the sample by performing a centrifuge spin of the sample plate (e.g. 3000 rcf for 5 minutes) before starting a new run. The debris may be flushed out of the chip by washing and re-priming the chip. See "LabChip Kit Essential Practices" on page 26 for instructions on how to wash and reprime the chip.

4 Check the O-rings on the top surface of the chip interface and clean if necessary.

LabChip Kit Essential Practices

To ensure proper assay performance, please follow the important handling practices described below.Failure to observe these guidelines may void the LabChip Kit product warranty.[1]

Note: *It is important to keep particulates out of the chip wells, channels and capillary. Many of the following guidelines are designed to keep the chips particulate-free.*

For assay and instrument troubleshooting, refer to the LabChip GX Touch Software Help file or call PerkinElmer Technical Support at 1-800-762-4000.

General

- Allow the chip, sample plate and all reagents to equilibrate to room temperature before use (approximately 20 to 30 minutes).
- Clean the O-rings in the chip interface weekly and the electrodes daily. Refer to the Instrument Users Guide Maintenance and Service section for procedures.
- Avoid use of powdered gloves. Use only non-powdered gloves when handling chips, reagents, sample plates, and when cleaning the instrument electrodes and electrode block.
- Calibrate laboratory pipettes regularly to ensure proper reagent dispensing.
- Only the PerkinElmer-supplied clean room cloth can be used on the chip to clean the detection window.
- Water used for chip preparation procedures must be 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent).
- Using the “Reverse Pipetting Technique” (described next) will help avoid introducing bubbles into the chip when pipetting the gel.

1. PerkinElmer, Inc. warrants that the LabChip Kit meets specification at the time of shipment, and is free from defects in material and workmanship. LabChip Kits are warranted for 90 days from the date of shipment. All claims under this warranty must be made within thirty days of the discovery of the defect.

Reverse Pipetting Technique

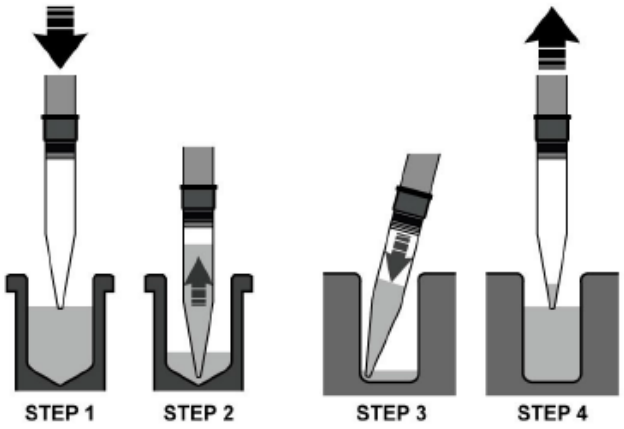

Figure 23. Reverse pipetting.

1. Depress the pipette plunger to the second stop.
2. Aspirate the selected volume plus an excess amount from the tube.
3. Dispense the selected volume into the corner of the well by depressing plunger to the first stop.
4. Withdraw the pipette from the well.

Reagents

- Store reagents at 4°C when not in use.
- The LabChip dye contains DMSO and should be thawed completely before use. It is recommended that you prepare aliquots to reduce the time required for thawing.
- Gently vortex all kit reagents before use.
- Dispense reagents into chip wells slowly without introducing air bubbles. Insert the pipette tip vertically and to the bottom of the chip well.
- Protect the dye and Gel-Dye mixture from light. Store in the dark at 4°C when not in use.
- The Gel-Dye mixture expires 3 weeks after preparation.

Chips

Repriming Chips

Note: *Buffer tubes filled with 1X sample buffer or water should be placed into the instrument while priming or washing chips.*

- Touch the *Unload Chip* button on the *Home* screen to open the instrument door. Place the chip into the instrument.
- Close the chip door securely and choose the corresponding assay.
- Touch the *Prime* button on the *Home* screen to reprime the chip.

Washing and Repriming Chips

- Touch the *Unload Chip* button on the *Home* screen to open the instrument door.
- Return the chip to the chip container ensuring the sipper is immersed in fluid.
- Thoroughly aspirate all fluid from the chip wells using a vacuum line.
- Ensure that each active well (1, 3, 4, 7, 8 and 10) is rinsed and completely aspirated twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
- Add 120 µL of Storage Buffer to each active well (1, 3, 4, 7, 8 and 10).
- Place the chip in the LabChip GX Touch/GXII Touch instrument.
- Close the chip door securely.
- Touch the *Wash* button on the *Home* screen (Figure 24).

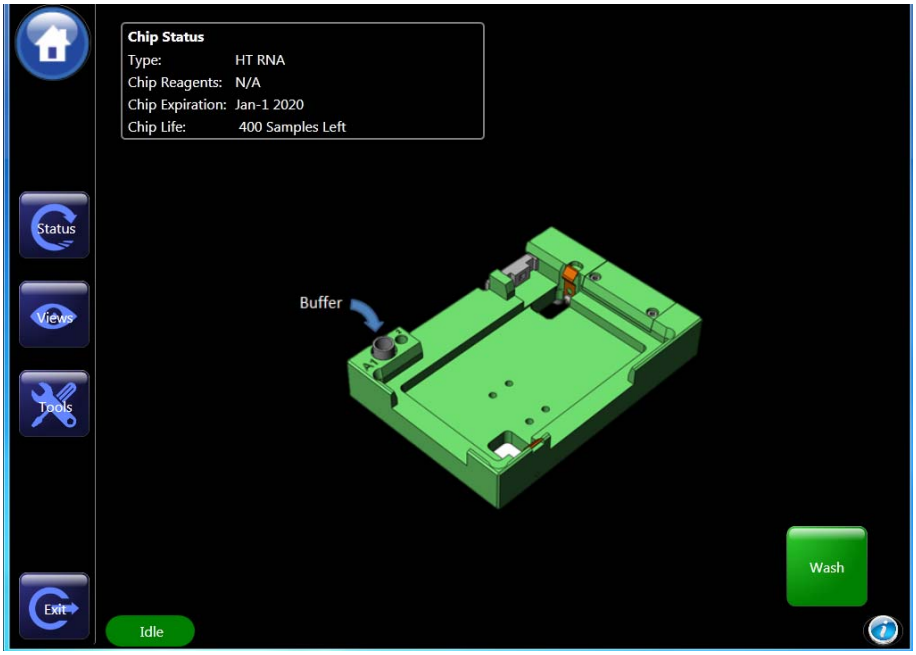

Figure 24. *Wash* screen.

- After the completion of the wash cycle return the chip to the chip container ensuring the sipper is immersed in fluid.
- Thoroughly aspirate all fluid from the chip wells using a vacuum line.
- Ensure that each active well (1, 3, 4, 7, 8 and 10) is rinsed and completely aspirated twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
- Add Gel-Dye solution to chip wells 3, 7, 8 and 10 using a Reverse Pipetting Technique as shown in Figure 23.
- Add 120 µL Marker (green cap ●) to chip well 4. Please note that the marker well may need to be replenished if the chip is in idle mode on the instrument for an extended period of time.
- Place the chip into the LabChip GX Touch/GXII Touch instrument.
- Close the chip door securely.
- Touch the *Run* or *Prime* button on the *Home* screen.
- If air bubbles are not dislodged after a reprime, apply a vacuum to the sipper. Perform this by filling all active wells with 100 µL of Chip Storage Buffer. Then suction the sipper with a vacuum line as shown in Figure 25 until droplets of fluid flow out from the sipper. When suctioning the sipper, be careful not to bend or break the sipper. To facilitate this, cut the end of the pipette tip attached to the vacuum line to widen the mouth.

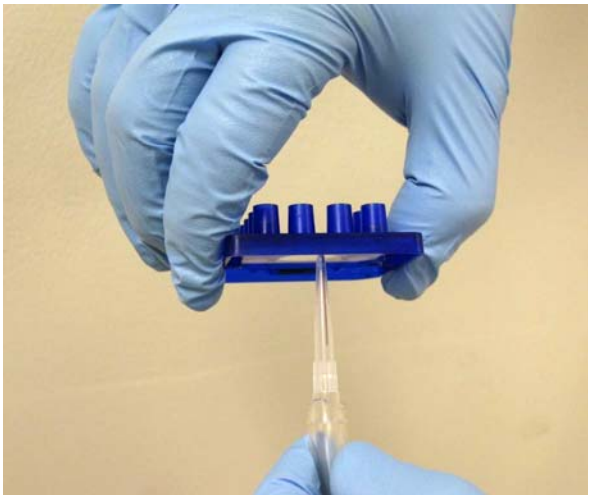

Figure 25. Removing an air bubble or clog by suctioning the sipper with a vacuum line.

Other Considerations:

- Chips should be stored refrigerated prior to first use.
- Cover the active wells on the chip with Parafilm® and store at 4°C. If using the chip again within 24 hours it may be left at room temperature.
- Do not allow the liquid in the chip container to freeze, as this may lead to poor chip performance. Do not submerge the chip in any solution.
- The entire chip surface must be thoroughly dry before use.
- The sipper must be kept immersed in fluid at all times and should not be exposed to an open environment for long periods of time.
- Use care in chip handling to prevent sipper damage. Damage to the sipper can result in inconsistent sampling.
- Avoid exposing the chips to dust by keeping them in a closed environment such as in the chip container or in the instrument before and after chip preparation.
- Chips can be prepared and left idle on the instrument for up to 8 hours. This workflow allows analysis of samples as needed throughout the day without having to re-prep the chip as long as the maximum number of samples per chip prep is not exceeded.
- PerkinElmer recommends the chip be re-prepared after it has been idle for 8 hours.

Samples

- Prepared sample plates should be free of gas bubbles and particulate debris, both of which may inhibit sipper flow.
- Sample plates containing gas bubbles and/or particulate debris should be spun down at 3000 rpm (1250 rcf) prior to analysis.
- Up to 96 samples in a 96-well or 384-well plate can be run with a single chip preparation.

Chip Well Aspiration Using a Vacuum

Aspirating with a pipette can leave used reagents in the chip wells. For this reason, PerkinElmer recommends vacuuming the wells instead. This can be accomplished by attaching a permanent pipette tip to a house vacuum line with trap (Figure 26). To avoid contamination, use a new disposable pipette tip over the permanent tip for each chip aspirated (Figure 27).

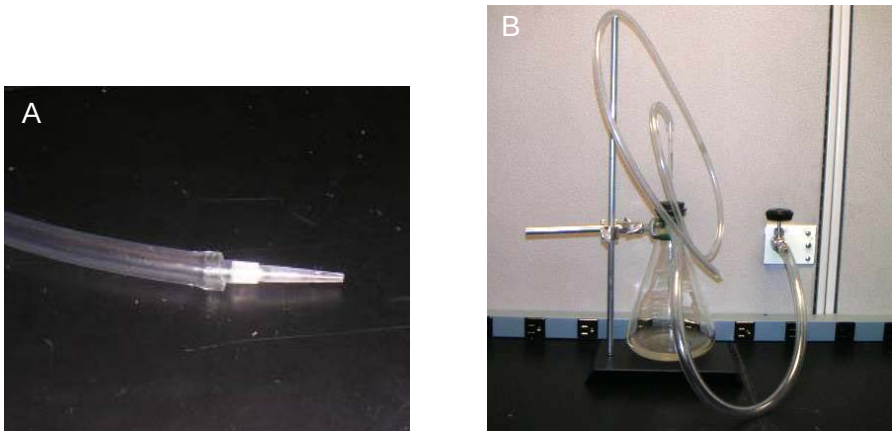

Figure 26. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap.

Figure 27. Replacing the disposable pipette tip.

Reordering Information

Table 6. Reordering Information

Product	Catalog Number
DNA 5K/RNA/CZE LabChip for LabChip GX Touch/GXII Touch HT	760435
DNA 5K/RNA/CZE LabChip for LabChip GX Touch/GXII Touch 24	CLS138949
RNA Reagent Kit	760410
Buffer Tubes	E&K Scientific, Cat. # 697075-NC
Ladder Tubes	Genemate, Cat. # C-3258-1
2.0 mL Centrifuge Tubes	Eppendorf Cat. # 022363352,

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Email: global.techsupport@perkinelmer.com
Internet: www.perkinelmer.com

For additional assay and instrument troubleshooting, refer to the LabChip GX Touch Software Help file.

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